



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM:

To: Autumn Metzger

From: Kevin Sweeney, Senior Entomologist

A handwritten signature in black ink, reading "Kevin J. Sweeney".

Through: Pesticide Evaluation Review Committee (PERC)

Handwritten initials in black ink, possibly "L M W".

Date: June 11, 2015

Subject: PRODUCT PERFORMANCE DATA EVALUATION RECORD

This is a primary review of a rebuttal containing additional efficacy data for this registration action.

DP barcodes: 426808 and 426809

Decision Nos.: 494935 and 494679

Submission Nos: 965920 and 965921

Action code: R340

Product Names: Hartz Reference #143 and #144

EPA Reg. Nos: 2596-178 and 2596-179

Formulation Type: pet spot-ons

Ingredients statement from the label with PC codes includes:

2596-178 = 9.8% fipronil, pc code 129121; 0.25% pyriproxyfen, pc code 129032; and 11.80% (S)-methoprene, pc code 105402.

2596-179 = 9.8% fipronil, pc code 129121; 0.25% pyriproxyfen, pc code 129032; and 11.80% (S)-methoprene, pc code 105402.

Application rate(s) of product: linear dose increments depending upon the weight range as directed by the label.

2596-178 = product dose range from lowest weight class is 0.67 ml (0.023 fl. oz.) (5 lbs) to 4.02 ml (0.136 fl. oz.) (132 lbs.) for the highest weight class.

2596-179 = 1.5 ml (0.051 fl. oz.) applied to cats weighing 3 lbs or more.

Use pattern:

2596-178 is a spot-on for dogs. Apply in a continuous band along the dorsal midline from head to tail.

2596-179 is a spot-on for cats. Apply in a single spot between the shoulder blades on the back of the cat.

OCSPP/OPPTS Guidelines: OPPTS 810.3300.

I. Action Requested: Review raw study report data associated with previously cited and reviewed public literature in support of a label amendment. Registrant has requested that the following claims be added to the labels of both products:

“Controls flea infestations on treated cats (and kittens) (&kittens) and prevent infestations in the home.”

“Prevents fleas on treated cats (and kittens) (&kittens) from infesting (reinfesting) your home”

II. Background: The cited literature (nine articles) presented data on a variety of insecticide combinations applied as a spot on to pets. Hartz wishes to use those data to support the above claims, which indicate that applying their product to a pet will control /prevent flea infestations in the home/indoor premises. EPA and Hartz met on October 14, 2014 to discuss these claims and the data needed to support them. Hartz presented a discussion on a new study to EPA and EPA provided input on the study design during the meeting and in a protocol review. Concurrently, Hartz advised EPA that an amendment was pending that included literature to support the above label claims. Instead of relying on the proposed study results, they requested EPA to review the cited published literature to support the claims. EPA advised Hartz that public literature does not stand-alone, but may be used as part of a weight of the evidence approach to supplement a study testing the label claims for control and prevention of fleas in the home.

Subsequently, I reviewed the cited studies on January 8, 2015. I concluded that the cited studies were not sufficient to stand-alone in support of the proposed label claims. My recommendations included “... *The last three studies by Dryden were the most relevant citations but this published literature did not provide the raw data or meet the requirements of GLP. Furthermore, the studies were not conducted with the subject product or with a product that is substantially similar to the subject product.*” In response, Hartz requested – and was granted – an extension of the PRIA date during which time they submitted the study report for literature item 8 below in the attached Appendix.

III. Study Report Review:

Hartz submitted an original study report to supplement the literature cited in Item 8 (see below in Appendix) in order to provide raw data and document study findings. This study was conducted with two registered products (Frontline Plus) containing fipronil (insecticide/adulticide) and S-methoprene (IGR). The subject product contains fipronil (insecticide/adulticide), S-methoprene (IGR), and pyriproxyfen (IGR).

Study Report: VS-USA-28730

MRID49593901. Dryden, M., S. J. Gross, and D. Carithers. 2010. Evaluation of the Efficacy of Frontline Plus for the Control of Flea Populations and Flea-Gender Analysis in Heavily Flea Infested Private Residences and Populations on Naturally Infested Pets in

Tampa, Florida, 13 Years after Fipronil Introduction.

Background: In 1997, one year following product introduction, Frontline Top Spot was assessed in households in the Tampa area. Twelve years later in 2009 this study was performed with Frontline Plus to assess the continued efficacy of fipronil-based products against fleas in the Tampa, Florida area. In the interval between the two studies, retrospective observations were made on retained flea trap samples from household studies, noting that over time, the gender ratio of the flea population tended to shift from predominantly female (or at least 50:50) to predominately male as the flea infestation was being eliminated by on-animal flea control. This study was designed to examine and document these trends on a large scale to help evaluate the success of the flea control program with Frontline. (Note: EPA did issue an EUP for Top Spot in the late 1990s. Much of that data supported current Merial product uses.)

Purpose: determine the impact of appropriate on-animal treatments made to all pets in the household on premise flea populations, flea gender ratio, and on-animal flea burdens.

Materials and Methods:

Location: The study was conducted in Tampa, Florida.

Test substance/Product: fipronil (9.8% w/w)-(S)-methoprene (8.8% w/w) and cats were treated with fipronil (9.8% w/w)-(S)-methoprene (11.8% w/w) (Frontline®Plus for dogs and cats, EPA Reg. Nos. 65331-5 and -4, respectively.)

Test substance/Product application: All pets (dogs and cats) were weighed and Frontline Plus was applied to them according to label directions. Treatments were made on Day 0 and Day 28-30. No other topical or premise treatments were made during the study. More detail provided in treatments descriptions below.

Pest species: cat flea, *Ctenocephalides felis*.

Study design: This was a non-randomized, non-controlled, non-blinded, field study to evaluate the effect (impact) of Frontline Plus on flea emergence/control and flea gender ratios in heavily infested homes in the Tampa area.

Experimental unit: Each household was considered an experimental unit for site assessment of flea emergence and gender ratios. Each animal was considered an experimental unit for on-animal flea assessments.

Animals: the pets (dogs and cats only) used in this study were homeowner owned. (Note: animals had to be qualified for the study as described in the criteria below. It appears that in some cases not all animals qualified.)

Criteria for household inclusion: 29 homes and their respective pets were selected in the Tampa area in the summer of 2009. The inclusion criteria were:

1. Fleas present on pets (minimum of five fleas).
2. Fleas present in home (based upon on at least 5 fleas in KSU intermittent flea light traps).
3. One to four healthy dogs or cats (no more than four).
4. Owner agreed to participate for at least two months.
5. Owners willing to not use any other premise or topical flea treatment during the study.
6. Owner signed consent forms.
7. Owners understood there were no restrictions on the animals regarding exposure to rain, swimming or movement outdoors.
8. No mammalian pets other than dogs or cats in the household.

On-Animal Product Treatments: Dogs were treated topically according to label dosing recommendations; the entire dose was applied on one spot between the shoulder blades for dogs or on the neck for cats. Pets were weighed on Days -1 or 0 to ensure proper dosing. All pets in each enrolled household were treated on Day 0 and then once between Days 28-30. All treatments were applied by study investigators. No other topical or premise flea treatments were used during the study. There were no restrictions on the animals with regard to exposure to rain, swimming or movement outdoors. However, while pet activity was not be restricted, it was recorded. It should be noted that in some homes with multiple dogs and/or cats not all pets qualified for inclusion in the study (fewer than 5 fleas, inability to examine, etc). However, all qualifying and non-qualifying dogs and cats within each enrolled household were still administered weight/species appropriate treatment.

Premise Flea Population Assessment: The numbers of adult fleas emerging in the home were assessed using intermittent light traps¹. Two traps were placed in two distinctly separate rooms/areas for 16 to 24-h for each household assessment period. Light trap site selection was based on where the pet(s) spend most of their time or where owners had observed fleas. Once trap locations were selected for a household, the traps were returned to those same locations for each subsequent counting period. Fleas collected on the adhesive pads of the traps were enumerated and identified as to characteristics (e.g.; fed, unfed, gravid) and as to species. In addition, the gender of fleas collected by light traps for each counting period, for each household, was determined. Environment flea counts were conducted + 1 day on Days 0, 7, 14, 21, then once between Days 28-30, 40-45, and 54-60.

On-Animal Flea Population Assessments: The flea population densities on each pet were estimated using a visual area count methodology (hand counts)². Area counts were performed at five locations on each animal; dorsal midline, tail head, left lateral, right lateral and inguinal region. Area counts were limited to one minute per location and conducted by parting the hair against the lay using both hands until the selected area was covered. Animal flea count estimates were conducted + 1 day on Days 0, 7, 14, 21, then once between Days 28-30, 40-45, and 54-60.

¹ Dryden M, Broce A. Development of a flea trap for collecting newly emerged *Ctenocephalides felis* (Siphonaptera: Pulicidae) in homes. J. Med. Entomol, 30:901-906, 1993.

² Dryden M, Boyer J, Smith V. Techniques for estimating on-animal populations of *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 31:631-624, 1994.

Flea in Premise) Control Assessments: Percentage of control achieved by the flea products were calculated using Geometric Means (GM) and the following formula:

$$\frac{(\text{Day } z \text{ GM Flea trap Counts} - \text{Day } y \text{ GM Flea trap Counts})}{\text{Day } z \text{ GM Flea trap Counts}} \times 100 = \% \text{ control}$$

Where z = Day 0, or the Day with the highest number of fleas collected (red-line homes)

Where y = Day 54-60 (completion date of household)

To determine these geometric means, counts were transformed to the natural logarithm of (count + 1) for calculation.

There were also a subset of homes where an increase in trap counts of at least 125% over Day 0 counts was seen within thirty days following product application on Day 0 (identified in data tables as red-line homes). To address this issue, a second measure for control assessment was performed. In these analysis, using the formula noted, the highest trap total count seen in the first 4 weeks for each household (not necessarily Day 0) was compared to the final trap count for that household. These assessments were performed including all fleas found in the flea traps - fed or unfed. The Geometric Mean Flea Counts of unfed fleas captured in these traps were assessed in the same manner as total fleas captured in the trap. Day 0 to final count, and highest count to final count.

Gender-Ratio Assessments: Fleas collected in traps were assessed for gender throughout the study, noting the arithmetic ratio of female to male fleas (f:m) for each study site. Additionally, arithmetic ratios of only unfed fleas found in the traps were assessed.

On-Animal Flea Counts: On-animal flea count estimates of live adult fleas were transformed to the natural logarithm of (count + 1) for calculation of geometric means by assessment group at each time point. Percent reduction from the control (Day 0) mean were calculated using the formula $[(C - T) / C] \times 100$, where C = geometric mean for the control count (Day 0) count and T = geometric mean for the treated group for each subsequent assessment (Days 0, 7, 14, 21, then once between Days 28-30, 40-45, and 54-60).

Statistics:

Data transformation: As discussed above, this study transformed count data. Count data (e.g., number of individuals or species) are often log-transformed to satisfy parametric test assumptions. Apart from the fact that generalized linear models are better suited in dealing with count data, a log-transformation of counts has the additional quandary in how to deal with zero observations. With just one zero observation (if this observation represents a sampling unit), the whole dataset needs to be "adjusted" by adding a value (usually 1) before transformation.

Flea control/reduction assessment: To compare the reductions in flea counts in traps from Day 0 to Day 54-60 and from "maximum count to Day 54-60, the final count value was subtracted from the starting value, and the difference was transformed to the natural logarithm of (count-+)

1): if the final count was higher than the initial count, causing a negative "reduction" the difference was defined as equal to zero. A t-test was then performed on the transformed differences with a null hypothesis that the mean difference was equal to zero; 2-sided p-values were reported.

Gender ratio: To compare the sex ratio of all fleas or unfed fleas in traps on Day 6 to that on Day 54-60, a test of two independent proportions was used; 2-sided p-values were reported. Flea counts on the pets were summarized for each site by calculating geometric means for each site and day as described for trap counts; maximum count for each animal or each site was not determined. Geometric means overall and for each type of site were calculated as described for trap counts, with each site having equal weight in the calculation, and percent reduction from Day 0 to Day 54-60 was calculated as described above for trap counts. The p-value for the difference between Day 0 and Day 54-60 flea counts was determined the same as for trap counts.

Study Results:

Data from twenty-seven households were analyzed in these assessments. Premise flea control and flea gender-ratio assessments were calculated based upon the total numbers of fleas found in the flea traps, and separately by the number of unfed fleas present in the flea traps. On animal flea counts were based on total estimates.

The authors presented data from a 60 day EUP-like field study where every animal (dog and/or cat) received two monthly treatments of FrontLine products. The report focused on the impact (measured by % reduction based on geometric mean counts from flea trap collections) of these treatments on premise flea populations in areas most commonly occupied by the pets such as a resting or sleeping site. Second, the effect of these treatments on flea gender ratios and blood feeding was examined. On-animal counts were also reported. Data were presented in table form with raw data reports located in appendices. The authors also provided special consideration to the most heavily infested households where flea counts actually increased at the beginning of the study.

Flea trap counts for the total fleas captured for each collection date were converted to the geometric mean and the percent control was calculated based on the geometric mean counts seen in the traps at Day 0. By the end of the study (Day 54-60), the percent reduction from Day 0 flea trap counts was 89.2%, with homes described as red-line homes (sites where the count increased by >25% within the first 30 days) experiencing 77.3% reduction and normal homes (sites where the count did not rise over 25% within the first 30 days) a 94.9% reduction of flea burdens was seen (Table 2). In all cases, the reductions seen were all statistically significant ($p < 0.05$). When percent reduction at the end of the study was calculated based upon the geometric mean of the final count against the geometric mean of the highest flea trap burden measured within the first 30 days of the study, the efficacy for all households was 93.3%, with the red-line home reduction at 91.8% and a reduction of 95.1% was seen in the normal homes (Table 2). These reductions too, were all statistically significant ($p < 0.05$). These same assessments were measured, only counting fleas that were unfed and in the traps, excluding any fleas that had taken a blood meal. When measuring reductions from Day 0 for unfed fleas the total was 85.4%, red-

line homes were 69.7% and normal homes were 93.1%. When reductions were measured against the high burden present in the first thirty days, the unfed flea total was 92.5%, red-line homes measured 90.9% and the normal homes were 94.5% (Table 3). Again, all reductions were statistically significant ($p < 0.05$).

Table 2. Geometric mean TOTAL flea (<i>Ctenocephalides felis</i>) trap counts and reduction from control (Day 0) and reduction from maximum trap count, from infested households where all dogs and/or cats are treated with FRONTLINE Plus									
	Day 0	Day 7	Day 14	Day 21	Day 28-30	Day 40-45	Day 54-60	% reduction from Day 0	% reduction from maximum trap count'
All Homes	13.5	6.9	9.8	6.8	4	2.8	1.5	89.20%*	93.30%*
-red line homes	11.9	10.5	17.3	10.8	5.1	5.1	2.7	77.30%*	91.80%*
-normal homes	14.9	4.8	6.1	4.6	3.2	1.6	0.8	94.90%*	95.10%*

*Reduction observed was statistically significant ($p < 0.05$)

I reviewed the raw data presented in this study report and calculated the mean flea count for each day for each group of homes as presented in Table 2a below. The % reduction values for all homes is based on a weighted average because there were 12 red line homes and 15 normal homes. The arithmetic means tends to be more sensitive to outliers as evidenced by the data reported in days 40-45 where one home had 267 fleas. The geometric mean provides a measure more like a median value or one of central tendency.

Table 2 a. (Reviewer calculated from raw data). **Arithmetic mean** of TOTAL flea (*Ctenocephalides felis*) trap counts and reduction from control (Day 0) and reduction from maximum trap count, from infested households where all dogs and/or cats are treated with FRONTLINE Plus.

	Day 0	Day 7	Day 14	Day 21	Days 28-30	Days 40-45	Days 54-60	%reduction from Day 0 at days 54-60	% reduction from maximum trap count +
All Homes								83.6	85
-red line homes only (12)	13.25	17.6	29.8	20.58	7.5	26.6***	3.83	72%	70%
-normal homes only (15)	20.26	8.4	8.4	7.6	4.73	2.8	1.6	93%	97%

*** Home # 7 had 267 fleas collected. This home had one pet.

+ Mean highest infestation day through day 30 is 42.5. The highest average infestation count occurred most frequently on Day 14.

Table 3. Geometric mean *UNFED* flea (*Ctenocephalides felis*) trap counts and reduction from control (Day 0) and reduction from maximum trap count, from infested households where all dogs and/or cats are treated with FRONTLINE Plus

	Day 0	Day 7	Day 14	Day 21	Day 28-30	Day 40-45	Day 54-60	% reduction from Day 0	% reduction from maximum trap count
All Homes (27)	6.7	3.8	6.3	4.9	2.4	2.4	1.0	85.4%*	92.5%*
-red line homes only (12)	5.8	5.6	12.0	7.9	3.0	4.3	1.7	69.7%*	90.9%*
-normal homes only (15)	7.6	2.7	3.7	3.3	2.0	1.4	0.5	93.1%*	94.5%*

*Reduction observed was statistically significant ($p < 0.05$)

Gender-Ratio Assessments: The total number of fleas captured in the flea traps were assessed for gender and results recorded. Using these data the gender distribution for the total flea population present in the flea traps was assessed. In all homes, at and near initiation of the study, the gender most represented in the capture traps were female fleas, representing 57.9-66.5% of the captured flea population. By the end of the study, the female fleas represented only 42.3% of the captured flea population. This same trend was seen when the houses were segregated into the red-line homes (females representing 67.3%-70.8% in initial counts, falling to 37.0% by study end). These reductions for the total homes and redline homes were statistically significant ($p < 0.05$). Although less apparent, a gender shift was still seen in the normal homes (53% falling to 35.7% by day 40-45, however this percentage rose by study end due in part to the low infestation levels and presence of fed female fleas) (Table 4). However, when these same assessments were measured, counting only fleas that were unfed and in the traps, excluding any fleas that had taken a blood meal, the gender-shift seen on all homes and red-line homes, when counting unfed fleas were statistically significant ($p < 0.05$). In all homes, at and near initiation, the gender distribution for the unfed flea population was weighted toward the female fleas, representing 57.5% to 59.3% of the captured flea population. By the end of the study, female flea representation fell to only 25% of the total population captured. This same trend was more pronounced in the red-line homes, starting out a bit higher at 62%-65.8% and finishing the study a bit lower at 21.4% of the captured population, and slightly less pronounced in the normal homes (53% falling to 33%), and not a significant change ($P = 0.179$) in these homes (Table 5).

Table 4. Percentage of TOTAL flea (*Ctenocephalides felis*) population comprised by female fleas, in flea traps placed in infested households where all dogs and/or cats were treated with FRONTLINE Plus

	% female fleas of total fleas collected						
	Day 0	Day 7	Day 14	Day 21	Day 28-30	Day 40-45	Day 54-60
All Homes	57.9%	66.5%	50.6%	39.9%	54.1%	42.8%	42.3%*

-red line homes only	67.3%	70.8%	47.8%	36.3%	56.9%	43.8%	37.0%*
-normal homes only	53.0%	58.6%	58.7%	42.4%	47.8%	35.7%	52.0%*

*Reduction observed from Day 0 to Day 54-60, was statistically significant ($p < 0.05$)

Table 5. Percentage of UNFED flea (*Ctenocephalides felis*) population comprised by female fleas, in flea traps placed in infested households where all dogs and/or cats were treated with FRONTLINE Plus

	% female fleas of total fleas collected						
	Day 0	Day 7	Day 14	Day 21	Day 28-30	Day 40-45	Day 54-60
All Homes	57.5%	59.3%	44.7%	32.2%	41.4%	40.0%	25.0%*
-red line homes only	65.8%	62.0%	42.5%	26.8%	42.0%	41.2%	21.4%*
-normal homes only	53.4%	53.8%	51.3%	39.0%	40.8%	30.8%	33.3%*

*Reduction observed from Day 0 to Day 54-60, was statistically significant ($p < 0.05$)

On-Animal Flea Counts: Most homes had one pet but some had as many as 3-4. The number of fleas on each pet for each reporting period was reported but whether a pet was a cat or dog was not reported and cat and dog data were not reported separately or analyzed. Instead data were pooled per household for overall calculation of the geometric means for every reporting period for every household. The red-line homes had a total of 23 pets while the normal homes had a total of 27 pets. The all homes data are based on flea collected from a sample size of 50 pets across 27 households. Normal homes showed the most consistent decreases in flea counts after the second product application through 60 days. The red-line homes data were less consistent and the flea load per animal did not differ for some of the animals, which may be due to picking up more fleas outside at these households. Outdoor flea counts were not performed.

Based on the data reported in the appendix, the on-animal flea count estimates for each count date were converted to the geometric mean and the percent control was calculated based on the geometric mean counts seen on animals at Day 0. By the end of the study (Day 54-60), the percent reduction from Day 0 counts for pets from all households in the study was 87.5%. The percent reduction in counts for pets in homes described as red-line homes (sites where the household trap counts increased by 25% within the first 30 days) experiencing 69.7% reduction and normal homes (sites where the trap counts did not rise over 25% within the first 30 days) a 95.0% reduction of on animal flea burdens was seen. All geometric mean count reductions from Day 0 to Day 54-60 were statistically significant ($p < 0.05$). The data show that there was an initial reduction in flea counts on the animals.

IV. Entomologist's Conclusions: The data provided showed that appropriate (monthly) on-animal treatments with Frontline Plus to a total of 50 dogs and cats reduced – but did not eliminate – premise flea populations by 60 days in 27 homes. The treatments results in 82% or greater control of fleas based on arithmetic means and nearly 90% if the assessment is based on the geometric means. Twelve houses reduced fleas by less than these values while 15 houses were better. These treatments also affected the gender ratio of female to male fleas, which resulted in a smaller population of females. On-animal treatments were slightly better than

expected in some homes and excellent in others. Geometric means were calculated in this study because there were no control treatments as these were EUP like trials in human occupied homes where they wanted to eliminate fleas. The products used in this study were not the same as the subject product and neither product contained pyriproxyfen. However, the products used in this test contained an equal or lesser amount of S-methoprene and the same amount of fipronil as the subject product.

V. Entomologist's Recommendations:

1. The data were submitted to amend the subject labels in order to add the following claims:

“Controls flea infestations on treated cats/dogs (and kittens/puppies) (&kittens/puppies) and prevent infestations in the home.”

“Prevents fleas on treated cats/dogs (and kittens/puppies) (&kittens/puppies) from infesting (reinfesting) your home”

The study results indicate that the on-animal product treatments of Frontline Plus:

- Reduced flea premise populations. The data had higher variability in the red-line homes compared to the normal homes.
- Controlled flea infestations on most treated pets. Dogs and cats were not identified but the claim is supported based on the wealth of data on these and related products.

2. Based on the above, the claims “Prevents infestations in the home” and “Prevents fleas from infesting (reinfesting) your home” are not supported. There was considerable variability in the results and mean fleas counts indicate that fleas were not eliminated in the treatments.

APPENDIX - For PERC review I attached the results of the first review below.

Reviews and recommendations from DER dated January 8, 2015.

The registrant submitted nine public literature citations in one MRID that were reviewed as follows on January 8, 2015.

MRID49455301. Volume 1: Supplemental Information - White Paper. "Submission of Laboratory and Field Studies to Confirm Topical Parasiticide Applications Control Pre-Existing In-Home Flea Infestations and Specific Support of In-Home Infestation Control Claims for EPA Reg. Nos. 2596-178 and -179."

The white paper included the following citations. I included the registrants' explanations from their white paper.

"1. Jacobs, D. E., M. J. Hutchinson, D. Ewald-Hamm. 2000. Inhibition of immature *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) development in the immediate environment of cats treated with imidacloprid. *Journal of Medical Entomology* 37 (2): 228-230.

Jacobs et al. (2000) showed that treated cats had transferred sufficient imidacloprid residues to blankets after a 6 hour contact per day, for 5 days. A new blanket was used each week for 4 weeks. Pre-adult flea viability on blankets was reduced by 100%, 84%, 60% and 74% in the 1st, 2nd, 3rd, and 4th week after treatment, respectively.

Entomologist's Conclusion: Unacceptable. The product tested was not similar to the subject product.

2. Jacobs, D. E., M. J. Hutchinson, D. Stanneck. 2001. Accumulation and persistence of flea larvicidal activity in the immediate environment of cats treated with imidacloprid. *Medical and Veterinary Entomology* 15: 342-345.

In another study, Jacobs et al. (2001) used the same test design except for increasing the contact periods to 10 or 20 6-hour blanket contact periods over 2 and 4 weeks, respectively. Results showed that the degree of control was significantly higher after 26 days achieving pre-adult flea control of 100% and 97.4% after 10 or 20 days of blanket exposure, respectively.

Entomologist's Conclusion: Unacceptable. This study was conducted with an imidacloprid based formulation that was not similar to the subject product.

3. Ross, D. H., R. G. Arther, C. von Simson, V. Doyle, M. W. Dryden. 2012. Evaluation of the efficacy of topically administered imidacloprid + pyriproxyfen and orally administered spinosad against cat fleas (*Ctenocephalides felis*): Impact of treated dogs on flea life stages in a simulated home environment. *Parasites & Vectors* 5:192.

Ross et al. (2012) evaluated pre-adult flea control of an orally administered ectoparasiticide versus a topical imidacloprid/pyriproxyfen application. Dogs were housed in pens with floor mats that could be sampled for flea development. Flea infestations were established on-animal 1, 16 and 21 days prior to ectoparasiticide administration. Results showed topical application of the imidacloprid/pyriproxyfen formula resulted in transfer of active ingredients to floor matting. After only 1 week of exposure to treated dogs, floor mat samples provided better than 95% control of adult flea emergence.

While laboratory studies have shown the ability of treated pets to transfer effective amounts of active ingredient within simulated home environments, the same transfer of parasiticide active ingredients under in-home conditions has been demonstrated through field testing.

Entomologist's Conclusion: Unacceptable. This study was conducted with a formulation that contained spinosad.

4. Dryden, M. W., H. Perez, and D. Ulitchny. 1999. Control of fleas on pets and in homes by use of imidacloprid or lufenuron and a pyrethrin spray. JAVMA 215 (1):39-39.

Dryden et al. (1999) investigated existing household flea infestations. The ability for topical parasiticides and oral parasiticides to control in-home infestations was evaluated. It is critical to note that other flea treatments, either applied to the pets or their surrounds, were not used during the study. As well, no restrictions were made regarding exposure of pets to rain, swimming and outdoor activity. Results showed the incidence of fleas on pets and pre-adult flea populations living in the home were reduced by the topical parasiticide application containing imidacloprid. On-animal flea control was achieved within 1 week and in-home infestation was reduced by 86.8% after 4 weeks.

Entomologist's Conclusion: Unacceptable. The product tested was not similar to the subject product as it contained an IGR and adulticides not present in this formulation.

5. Dryden, M. W., J. M. Denehberg, S. Bunch. 2000. Control of fleas on naturally infested dogs and cats and in private residences with topical spot applications of fipronil or imidacloprid. Vet Parasitol. 93: 69-75.

In a follow up study, both fipronil and imidacloprid were evaluated for on-animal and infestation control potential. The same conditions existed for no other pesticide treatments being during the test as well as no restrictions on pets' activities or movement. Results showed on-animal control was achieved for both active ingredients after 1 week. In-home infestation control was as well achieved for both active ingredients exceeding 89.0% after 4 weeks. (Dryden et al. 2000).

Infestation control for both of these studies confirms transfer of active ingredients from treated the in-home environment to control pre-adult flea infestations.

Entomologist's Conclusion: Supplemental. One of the adulticides, fipronil, is present in the formulation at the same level but a complete data set was not provided. Furthermore, the author relied on visual hand counts for assessing flea infestation levels on the animal instead of comb counts.

6. Maynard, L. P. Houffschmitt, and B. Lebreux. 2001. Field efficacy of a 10 percent pyriproxyfen spot-on for the prevention of flea infestations on cats. Journal of Small Animal Practice 42: 491-494.

Maynard et al. (2001) evaluated an IGR only application and the effects it had on in-home flea infestations. Topical application of a 10% pyriproxyfen formula resulted in flea infestation control, measured by 'zero-flea' cats, increasing through the study from 49% control on day 30 to 88% control on day 180. In this study, no restrictions were placed on pets' activities including movement outdoors. It was concluded that appropriately timed topical applications of pyriproxyfen offer a method of flea control in the domestic environment.

Entomologist's Conclusion: Unacceptable. The formulation is not similar to the subject product and amount of pyriproxyfen applied to the cats is not equivalent to the dose to be delivered by the subject product.

7. Dryden, M. W., P. Payne, v. Smith, B. Riggs, J. Davenport, and D. Kobuszewski. 2011a. Efficacy of dinotefuran-pyriproxyfen-permethrin and fipronil-(S)-methoprene topical spot-on formulations to control flea populations in naturally infested pets and private residences in Tampa, FL. Vet. Parasitol. 182:281-286.

Dryden et al. (2011a) repeated an in-home field trial methodology to investigate a dinotefuran/pyriproxyfen/permethrin topical formula versus topically applied fipronil/(S)-methoprene. The fipronil/(S)-methoprene formula provided in-home pre-adult infestation control greater than 88% after 4 weeks. Greater than 96% was achieved after 6 and 8 weeks showing progressive in-home infestation control over time with monthly treatments.

Entomologist's Conclusion: Supplemental. The data from the dinotefuran-pyriproxyfen-permethrin formulation are not relevant to this amendment. The data from the fipronil-methoprene combination products provide supporting data that may be useful in a weight of the evidence approach as the concentration of fipronil and methoprene are similar to the subject product. However, these data cannot stand-alone as they are not complete data sets. The % control at 30 days is less than 90% (Study Table 1).

8. Dryden, M.W., D. Carithers, A. McBride, B. Riggs, L Smith, J. Davenport, V. Smith, P. Payne, S. Gross. 2011b. A comparison of flea control measurement methods for tracking flea populations in highly infested private residences in Tampa FL, following topical treatment of pets with Frontline® Plus(Fipronil/(S)-Methoprene). Intern J. Appl. Res. Vet. Med. 9:356-367.

Dryden et al. (2011b) made an assessment of extremely high in-home flea populations with reinfestation pressure from outside environments. A fipronil/(S)-methoprene formula was tested. Significant flea populations existed in the homes studied. Heavy pressure from outdoor wild animal sources existed. With this constant pressure, over the 60 day study, the percent reduction in unfed adult flea emergence from Day 0 and from maximum trap counts in all homes studied were 85.4% and 92.5%, respectively. Under these excessive test conditions, reduction in flea egg production and the transfer of ectoparasiticide active ingredients in the home by treated pets provided significant infestation control.

Entomologist's Conclusion: Supplemental. The product tested a Merial product containing a similar level of methoprene and fipronil. The data were not collected with the subject product and complete data sets are not available. This citation may be useful in a weight of the evidence approach to supplement a product specific study.

9. Dryden, M. W., P. Payne, V. Smith, M. Chwala, E. Jones, J. Davenport, G. Fadl, M. F. Martinez-Perez de Zeiders, K. Heaney, P. Ford, F. Sun. 2013. Evaluation of indoxacarb and fipronil (S)-methoprene topical spot-on formulations to control flea populations in naturally infested dogs and cats in private residences in Tampa FL. USA. Parasites & Vectors 6:366.

Dryden et al. (2013) also made comparison of formulas containing fipronil/(S)-methoprene versus indoxacarb. The study was done in naturally infested homes. Infestations were noted as the highest ever recorded in Tampa, Florida. Over 2 months, results showed the in-home pre-adult flea infestation control achieved for indoxacarb and fipronil/(S)-methoprene formulas were 97.7% and 84.6%, respectively.

Entomologist's Conclusion: Supplemental for reasons stated for the previous study.

Hartz Conclusions:

"All studies, both laboratory as well as field studies, document in-home pre-adult flea infestation control can be achieved by topical ectoparasiticide treatments. Specifically, laboratory testing under simulated home conditions have substantiated that effective residues of ectoparasiticide formulas do transfer from treated pets to surrounding surfaces. As seen from field trials, those residues in addition to the reduction

in viable eggs being laid on-animal are sufficient to provide pre-adult flea infestation control in the home even under severe adult flea re-infestation pressure.

As for the three field trials discussed above evaluating fipronil/(S)-methoprene topical formulas, and cats were treated with fipronil (9.8% w/w)-(S)-methoprene (8.8% w/w) and fipronil (9.8% w/w) -(S)-methoprene (11.8% w/w), respectively. Results showed flea infestations in the home environment were reduced by over 88% in 4 weeks, by over 92% from peak population counts over 2 months and by over 84% in 2 months. Even with constant heavy pressure from the outside environment, monthly re-applications of a fipronil/(S)-methoprene formula, and no secondary environmental pesticide applications, in-home flea infestations that existed prior to test product application were controlled over a 1 to 2 month period (Dryden et al. 2011a, 2011b, 2013).

These 3 field studies are submitted specifically in support of the claims,

"Controls flea infestations on treated (insert pet) and prevent infestations in the home."

"Prevents fleas on treated (insert pet) from infesting (reinfesting) your home." for EPA registration 2596-178 and 2596-179."

Entomologist's Conclusion: The Dryden et al. studies provide supplemental evidence that transfer of S-methoprene and fipronil may take place in the home. Light trap data were used to survey for fleas but visual hand-counts on the animal were also used. These studies do not meet GLP requirements and were not conducted under an Experimental Use Permit.

Entomologist's Recommendations:

1. The cited literature provides supplemental information on the ability of pet spot-on products to control/prevent fleas in the home environment. However, these citations do not stand alone. A GLP experiment must be conducted to show that this product performs as claimed. Therefore, the proposed claims are not acceptable based on citation of literature.

The last three studies by Dryden were the most relevant citations but this published literature did not provide the raw data or meet the requirements of GLP. Furthermore, the studies were not conducted with the subject product or with a product that is substantially similar to the subject product.